21. (Amended One Time) The transformed *Candida maltosa* strain of Claim 20 wherein the enhanced alkane hydroxylating activity of a) arises from DNA fragments encoding cytochrome P450 monooxygenase [ALK1-A] Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase [ALK3-A] Alk3-A (SEQ ID NO:37).

26. (Amended One Time) A DNA fragment comprising a) a first *Candida maltosa* PGK promoter which is operably linked to a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)) and b) a second *Candida maltosa* PGK promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 reductase.

REMARKS

Claims 1-27 are pending in this application. Claims 1-27 have been rejected. Claim 24 has been cancelled without prejudice. Claim 8 has been objected to and has been amended according to the Examiner's suggestion. Claims 4-6, 8-13, 16, 20-21 and 26 have been amended to more clearly define Applicants' invention.

The following remarks are numbered to correspond with the paragraph numbering of the pending Office Action. Applicants respectfully submit that no new matter has been entered by these amendments. Accordingly, Applicants request entry of all amendments and allowance of all claims.

3. Claim 8, part b has been amended to recite "C_{22}" rather than "C22".

Patentability Under 35 USC § 112:

4. Claims 4, 9, 11, 12, 20 and 26 are rejected under 35 U.S.C. 112, first paragraph, as improperly incorporating essential material by reference. Applicants addressed the rejection by inserting in the specification sequence listing that now includes data corresponding to the Gene Bank references contained in the original specification. Additionally, references to the appropriate sequence ID Nos. have been inserted in the text of the specification and in amended Claims 4-6, 11-13, 20-21 and 26. No new matter has been inserted and the amendments conform with the sequence rules of 37 CFR 1.821-1.825. Applicants have amended rejected claims by inserting the sequences to correspond to the referenced GenBank data. A paper copy, a computer readable disk and an appropriate declarations are attached. In light of these amendments, Applicants respectfully request reconsideration and withdrawal of these rejections and prompt allowance of the claims.

5. Claim 24 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants have deleted Claim 24 without prejudice in follow of Claim 25. In light of this amendment, Applicants respectfully request reconsideration and withdrawal of this rejection.

Patentability Under 35 USC § 102:

7. Claims 10, 11 and 24 are rejected under 35 U.S.C. 102(a) as being anticipated by Park et al. Park et al. teach simultaneous overexpression of ALK1 under the control of both GAL1 and GAL10 promoters on a single plasmid and they use *Candida maltosa* as the host cell. The Examiner states that Claims 10 and 24 are clearly anticipated by these teachings and Claim 11 is included because even though the specific accession numbers are not recited the ALK genes must be the same since they are *C. maltosa* genes. Applicants traverse these rejections.

A rejection under 35 USC § 102 requires that each and every element of the claimed invention be present in the cited reference. The examples reported by Park et al. are based on DNA maintained as replicating plasmids in *C. maltosa*. Plasmids are closed, circular pieces of DNA that are maintained separately from the chromosomes and may show instability when the cell replicates. In contrast, genes to be expressed in the instant invention are integrated into the cells genomic DNA, the chromosomes, which the cell naturally maintains upon replication.

The promoter used by Park et al. to control expression of the genes functions only under special nutritional conditions. As reported by Park et al. on page 27, column 2, paragraph 2, GAL1/10 promoters function only in the absence of glucose and the presence of galactose. Thus, only after cell growth on glucose has stopped (Park et al. page 25, column 2, paragraph 1), can galactose be added to allow gene expression. In contrast, the PGK promoter used in the instant invention functions in the presence of glucose. This attribute allows gene induction to occur while the cell is still actively growing and in a healthy nutritional state.

Applicants have amended Claim 10 to require that the genes be integrated. The Examiner is referred to page 18 (first paragraph) and Example 9 on page 35 of the instant invention, both of which clearly discuss integration of the DNA into the host genome.

Applicants have cancelled Claim 24. Claim 11 is dependent on Claim 10. In view of the amendment of Claim 10 and the foregoing discussion, Applicants respectfully request that the rejections for anticipation under 35 USC § 102(a) be withdrawn and Claims 10-11 be promptly allowed.

8. Claim 16 is rejected under 35 U.S.C. 102(b) as being clearly anticipated by Masuda et al. Masuda et al. teach a transformed *Candida maltosa* which has both the POX2 and POX4 genes disrupted. The Examiner states that this clearly anticipates Claim 16. Applicants have amended Claim 16 to specify that no more than both POX4 genes encoding acyl-CoA oxidase are disrupted in the *Candida maltosa* mutant.

Masuda et al. disclose genetic disruption of both POX2 genes and POX4 genes. Disruption of POX4 genes prevents the growth of *Candida maltosa* on tetradecane, n-tetradecanol and oleic acid. These substrates contain either a 14- or 18-carbon backbone. Masuda et al. do not disclose the ability of this mutant to grow on substrates other than tetradecane, n-tetradecanol and oleic acid that are also metabolized through the β-oxidation pathway. It is known that different acyl-CoA oxidase isozymes have distinct substrate chainlength specificity (Picataggio et al., *Mol. Cell. Biol.* 11:4333-4339 (1991)). *Candida maltosa* may have contained additional POX genes for degradation of substrates other than those disclosed by Masuda et al. Consequently, the materials of Masuda et al. do not anticipate a mutant with no more than disruption of both POX4 genes to block the β-oxidation pathway in the production of monoterminal and diterminal aliphatic carboxylates. Example 7 of the instant application supports this amendment and argument.

In view of the amendment and foregoing discussion, Applicants respectfully request that the rejection for anticipation under 35 U.S.C. § 102(b) be withdrawn and Claim 16 be promptly allowed.

9. Claims 24-26 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Zimmer et al. Zimmer et al. teach an expression vector containing both the *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase for expression in *S. cerevisiae*. The Examiner states that this clearly anticipates the above claims. Applicants traverse this rejection.

Once again, a rejection under 35 USC § 102 requires that each and every element of the claimed invention be present in the cited reference. Zimmer et al. teach that *C. maltosa* NADPH-cytochrome P450 reductase was coexpressed in *S. cerevisiae* with each of the three *C. maltosa* cytochrome P450 forms. Zimmer et al. used *S. cerevisiae* Gal10 promoter to express P450's in *S. cerevisiae*. Applicants use *C. maltosa* PGK promoter to express P450's in *C. maltosa*. These constructs are distinct from one another. Applicants respectfully request that the rejections for anticipation under 35 USC § 102(b) be withdrawn and Claims 24-26 be promptly allowed.

Patentability Under 35 U.S.C § 103:

11. Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cregg et al. in view of Zimmer et al. Cregg et al. teach how to express foreign genes in *Pichia pastoris* and the advantages of using *Pichia pastoris* as a host cell. Zimmer et al. teach an expression vector containing both the *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase for expression in *S. cerevisiae*. The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to place the expression vector of Zimmer et al. in *Pichia pastoris* as the host cell with the expectation of attaining the benefits taught by Cregg et al. Applicants traverse these rejection.

Zimmer et al. teach expression of *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase in *S. cerevisiae* by using the *S. cerevisiae* GAL10 promoter. Cregg et al. teach expression of foreign genes *Pichia pastoris* by using the *Pichia pastoris* AOX1 promoter. In addition, Cregg et al. 1) basically teach unpredictability from protein to protein; 2) do not teach or anticipate co-expression of two genes; and 3) do not teach expression leading to commercial application a long-felt and unsolved problem. Applicants note that the use of the *S. cerevisiae* GAL10 promoter for expression of foreign genes in *Pichia pastoris* is not taught by Cregg et al. nor is it suggested by Cregg et al.

The Examiner asserts that placing the expression vector of Zimmer et al. into *Pichia pastoris* would lead to the expectation of functional expression. However, the relevance of this assertion is unclear, as the instant invention does not claim *S. cerevisiae* GAL10 promoter driving *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase genes in *Pichia pastoris*. Rather, the claims as amended are drawn to *Pichia pastoris* AOX1 promoter driving *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase genes in *Pichia pastoris*.

Relevant to this, Ratner et al. (*Bio/Technology* 7:1129) teaches that expression systems are protein-specific. In other words, functional expression of gene X in system Y does not mean that other genes will functionally express in system Y, or that other systems will functionally express gene X. Even for one skilled in the art, it is not possible to predict functional expression of a given gene in a given system. Cregg et al. is a good example of this high uncertainty of heterologous protein expression specifically for *Pichia pastoris*. Table 1 (Cregg et al., page 905) shows expression levels ranging from 0.3 g/L to 12 g/L. The data clearly shows high variability from protein to protein, indicating the unpredictability of producing foreign proteins in this system. For example, with hepatitis B surface antigen, 0.3 g/L corresponds to 3% of protein, whereas with bovine lysozyme, 0.3 g/L corresponds to 60% of protein; with human epidermal growth factor, 80% of protein corresponds to 0.5 g/L, whereas with invertase, 80% of protein corresponds to 2.5 g/L.

In addition, Cregg et al. does not teach or suggest co-expression of more than one foreign gene in *Pichia pastoris*, which is what the instant invention claims. Even if it was obvious to try co-expressing the *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase genes in *Pichia pastoris*, it was not obvious that it would succeed for the reasons discussed. Furthermore, it was not obvious that it would succeed to the extent so as to be commercially useful, a long-felt and unsolved problem, which is also not taught by Cregg et al.

In view of the foregoing discussion, Applicants respectfully request that the rejections for obviousness under 35 U.S.C. § 103 be withdrawn and Claims 1-7 be promptly allowed.

12. Claims 8-23 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Picataggio et al. (5,254,466) in view of Picataggio et al. (1992), Masuda et al., Zimmer et al. and Schunck et al. (1989).

Picataggio et al. ('466) teach enhancement of long-chain mono and dicarboxylic acids by disruption of the β-oxidation pathway in *Candida tropicalis* by disruption of the POX4 genes.

Picataggio et al. (1992) teach enhanced production of long-chain dicarboxylic acids by transformation of Candida tropicalis host cells that have had their β-oxidation pathway blocked with genes encoding cytochrome P450 monooxygenase and P450 reductase. The Examiner states that Picataggio et al. did in *Candida tropicalis* exactly what the Applicants have now done *in Candida maltosa* .

Masuda et al. teach a transformed *Candida maltosa* which has both the POX2 and POX4 genes disrupted. The Examiner states that Masuda et al. did the same thing in *Candida maltosa* that Picataggio et al. did in *Candida tropicalis*.

Zimmer et al. teach an expression vector containing both the *Candida maltosa* cytochromes P450 and NADPH-cytochrome P450 reductase for expression in *S. cerevisiae*.

Schunck et al. (1989) provide the sequence of the alkane hydroxylating cytochrome P-450 of *Candida maltosa* and discuss the similarity between *Candida maltosa* and *Candida tropicalis*.

The Examiner states that it would have been obvious to enhance the production of long chain mono- and dicarboxylic acids in *Candida maltosa* by following the guidance provided by Picataggio et al. (1992) with the expectation of achieving the same result in *Candida maltosa*. Applicants respectively traverse these rejections.

Picataggio et al. (1992) teach disruption of both POX4 and both POX5 genes to block the β-oxidation pathway and enable efficient production of dicarboxylic acids by *Candida tropicalis*.

Applicants note that contemporary taxonomy relies heavily on phenotypic characterization for the definition of genera and species. Based on phenotypic characterization, alone, one might expect a close relationship between *Candida maltosa* and *Candida tropicalis*. However, comparisons of nucleic acid relatedness based on the reassociation kinetics of denatured genomic DNA mixtures provide a more precise means of measuring the relatedness of two organisms. *Candida maltosa* is recognized as a separate species distinct from *Candida tropicalis* on the basis of physiological, morphological and immunological properties and was clearly distinguished from *Candida tropicalis* by insignificant DNA reassociation kinetics (Meyer et al., *Arch. Microbiol.* 104:225-231 (1975); Kaneko et al., Taxonomic studies on a hydrocarbon-assimalting Candida strain., *Agric. Biol. Chem* 41:2269-2276 (1977); Kunze et al., Identifizierung zweier Candida maltosa-Stamme mittels DNA Reassoziation., *Z. Allg. Mikrobiol.* 24:607-613 (1984). Given that *Candida maltosa* and *Candida tropicalis* are recognized as distinct and separate microorganisms, none of the prior documents suggest how to enhance dicarboxylic acid production in *Candida maltosa* by blockage of its β-oxidation pathway. Given the differences in the molecular biology of *Candida maltosa* and *Candida tropicalis*, it was not obvious that the same genetic alterations would have the same physiological effects in both microorganisms.

The instant invention teaches that *Candida maltosa* POX4 mutant may produce dicarboxylic acids and further distinguishes *Candida maltosa* from *Candida tropicalis* in which disruptions of both POX4 and POX5 genes were required for efficient production of dicarboxylic acids. Disruption of both POX4 genes in *Candida tropicalis* was insufficient to enable efficient production of dicarboxylic acids. Based on Picataggio et al. (1992), one skilled in the art might expect the need to disrupt additional POX genes (i.e., POX5) to achieve efficient production of dicarboxylic acids in *Candida maltosa*.

Furthermore, Masuda et al. teach that genetic disruption of both POX4 genes prevents the growth of *Candida maltosa* on tetradecane, n-tetradecanol, and oleic acid. Masuda et al. do not teach the inability of this mutant to grow on other substrates that are also metabolized through the β-oxidation pathway. Picataggio et al. (*Mol. Cell. Biol.* 11:4333-4339 (1991)) teaches the distinct substrate chain-length specificity of different acyl-coA oxidases isozymes. *Candida maltosa* may have contained additional POX genes for degradation of substrates other than those taught by Masuda et al. Consequently, the results of Masuda et al. are insufficient to teach whether POX4 gene disruption alone is sufficient to result in a functional block of the β-oxidation pathway. The instant invention teaches conversion of alkane and fatty acid substrates to the corresponding dicarboxylic acids. As mentioned previously, Masuda et al. do not teach whether the POX4 mutant would convert tetradecane, n-tetradecanol, oleic acid or any other substrates to the corresponding dicarboxylic acids.

The Examiner states that "The expectation of success in using *Candida maltosa* as the host cell is provided by Schunck et al., who teach the similarity of *Candida maltosa* to *Candida tropicalis*". Schunck et al. teach the similarity of P450 gene sequences to those previously characterized in *Candida tropicalis*. To one skilled in the art, the degree of similarity between these genes is sufficient to assign functions to the genes from *Candida maltosa*, but is insufficient to establish the similarity of both microorganisms. *Candida maltosa* is clearly distinguished from *Candida tropicalis* on the basis of physiological, morphological, and immunological properties as well as DNA relatedness.

It is believed that all matters currently at issue have been addressed in this document. Should any matter remain unresolved, please contact the undersigned as indicated.

Respectfully submitted,

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